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(54) STEROID ALKALOID FORMULATION

(71) ARUBA (QLD.) PTY. LTD.
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(72) BILL ELLIOT CHAM, EDWIN HAROLD JOHN GERNS AND HENRY HAROLD GERNS

(74) SF

(57) Claim

1. A pharmaceutical formulation containing at least one active ingredient selected from the group consisting of solamargine, solasonine and mono- and di-saccharides of solasodine and the pharmaceutically acceptable salts thereof together with a pharmaceutically acceptable carrier *excluding simple solutions or suspensions of said active ingredient in water or common organic solvents.*

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-73

Form 10

COMPLETE SPECIFICATION

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57853/80

Class Int. Class

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LODGED AT SUB-OFFICE
28 APR 1980
Sydney

Complete Specification for the invention entitled :

"TREATMENT OF MAMMALS"

The following statement is a full description of this invention, including the best method of performing it known to me/us

FOR FINANCE TO VALUE
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The present invention relates to pharmaceutical compositions containing steroid alkaloids and therapeutic methods employing steroid alkaloids. The compositions of the invention have been found to be useful in chemotherapeutic treatment of basal cell carcinomas, squamous cell carcinomas, of skin inflammations of mycotic infections such as tinea, of non-malignant dermatitis such as psoriasis, of haemorrhoids and of acne, and for other cosmetic uses.

The chemotherapy of cancers (with drugs) goes back into antiquity. Early Egyptian papyruses describe the application of various mixtures of drugs to ulcerating skin tumors. However, the emergence of chemotherapy as an effective modality of cancer treatment in modern medicine is a relatively recent development. Emphasis on chemotherapy as a primary modality for therapy has also come about with the realisation that cancer is often a systemic disease where local forms of treatment are often inadequate.

Large numbers of compounds have been studied in the search for new drugs with improved therapeutic efficacy. As a result of these efforts, several different classes of chemotherapeutic agents have been identified. The availability of a variety of drugs with different mechanisms of action and with differing host toxicities has provided a new dimension for the role of chemotherapy in the treatment of cancer.

The alkylating agents which include nitrogen mustard, cyclophosphamide, chlorambucil, molphalan, and busulfan, are among the oldest and most established drugs used for the treatment of cancer.

Antimetabolic agents have been defined and comprise another class of anti-cancer drugs. Methotrexate, fluorouracil, mercaptopurine, thioguanine, and cytosine arabinoside are included in this class of potent anti-tumour drugs.

Yet another class of agents found to have significant anti-tumour activity are the antibiotics, products of living organisms that have profound anti-tumour properties. Among these are drugs such as antinomycin D, mitramycin, daunoribicin, mitomycin C, and adriamycin.

Folklore has provided a different type of chemotherapeutic agent, the vinca alkaloids. Vincristine and vinblastine (U.S. Patent 3 225 030 in 1965 to Eli Lilly), extracts of the periwinkle plant (Vinca Rosea), have become firmly established in the treatment of acute leukemia and other types of cancer.

The compositions of the present invention provide for another efficient chemotherapeutic agent for the treatment of basal cell carcinomas and squamous cell carcinomas, solar keratoses and keratocanthomas. The compounds employed in the present invention are derived from extracts of solanum sodomeum, known as apple of Sodom, and incorrectly as devil's apple. This plant bears fruit which is similar in shape to, but smaller than an apple. The fruit of the plant is generally considered to be poisonous.

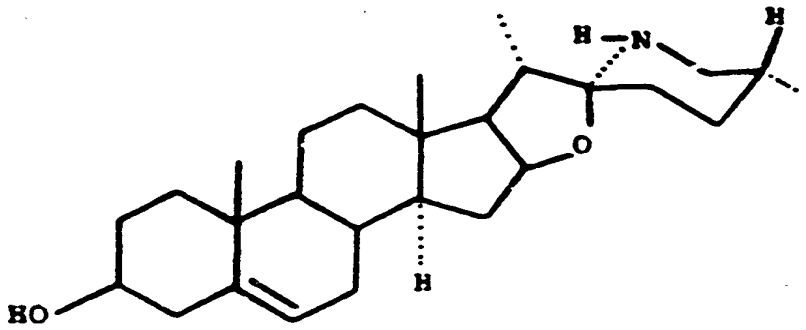
The present invention provides pharmaceutical formulations containing at least one active ingredient selected from the group consisting of solamargine, solasonine and mono- and di-saccharides of solasodine and the pharmaceutically acceptable salts thereof together with

a pharmaceutically acceptable carrier.

The method of the present invention comprises administering to a subject having the disorder to be treated an effective amount of the composition as outlined above.

Also within the scope of the present invention are processes for extracting steroid alkaloids from Solanum sodomeum.

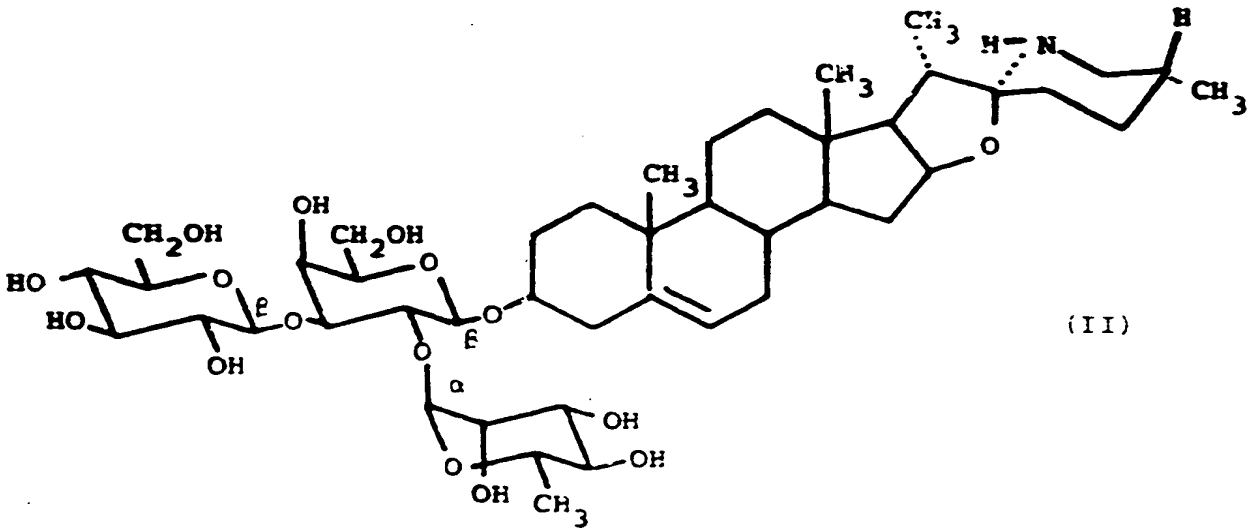
The aglycone isolated from Solanum sodomeum is:



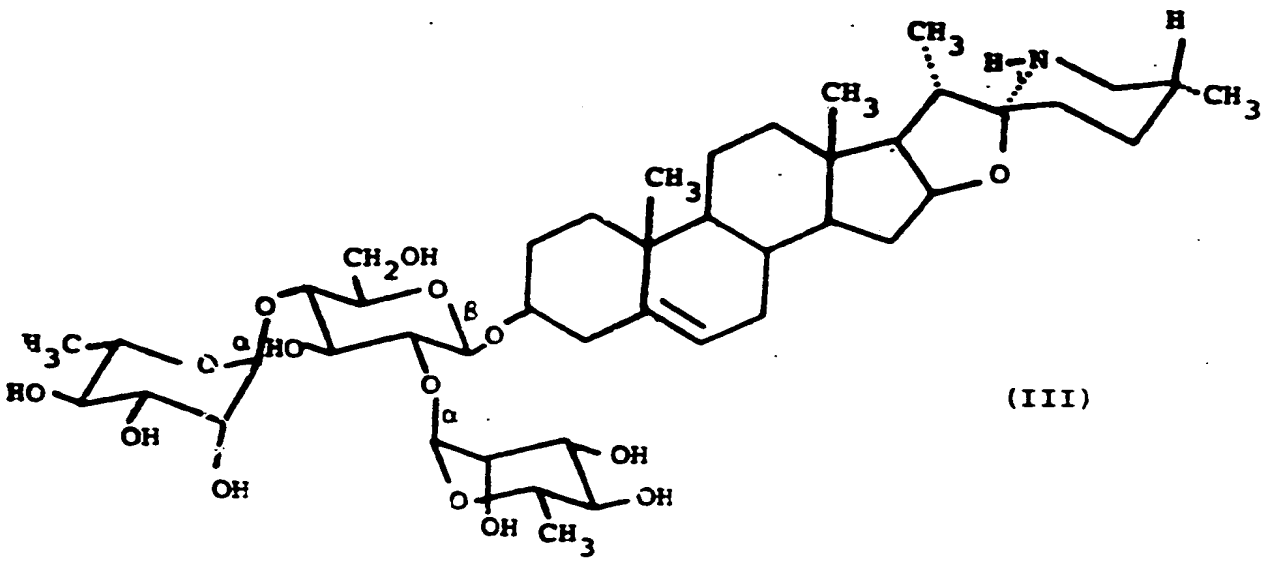
(I)

solasodine

The glycoalkaloids isolated from Solanum sodomeum are solasonine (II), solamargine (III) and mono- and disaccharides of solasodine (I)



(II)



(III)

The glycoalkaloids can be extracted from Solanum sodomeum by grinding any parts of the plant and subjecting the ground plant matter to the action of dilute acid, and making the acid extracts alkaline to precipitate the glycoalkaloids.

~~Thus the invention also provides a process for extracting glycoalkaloids from Solanum sodomeum comprising grinding parts of said plants subjecting the ground plant matter to the action of at least one dilute acid and making the acid extract alkaline to precipitate said glycoalkaloids.~~

The following examples illustrate extraction of a mixture of glycoalkaloids from Solanum sodomeum.

Example 1

Plant material from Solanum sodomeum is coarsely ground and then mixed with ten volumes (w/v) of 2% formic acid and shaken for two to four hours. This is then coarsely filtered (or centrifuged) and th residue is reshak n with ten volumes of 2% formic acid for another two to four hours at room temperature. The second xtract is

filtered and both solutions are added together and centrifuged to remove the last traces of residue. The solutions are made alkaline with ammonia, and heated to 80°C which causes a precipitate to form. This precipitate is dissolved in boiling alcohol and filtered while boiling. The alcohol is then evaporated to dryness. This yields a fine powder.

Example 2

Plant material from Solanum sodomeum is coarsely ground with two volumes (w/v) of 3% aqueous formic acid in a Waring blender. The mixture is diluted with another two volumes of 3% aqueous formic acid and is then shaken for eighteen to twenty hours at room temperature, then filtered through muslin. Two litre aliquots of the filtrate is heated to 50°C with continual stirring and then concentrated ammonia solution is added until the pH-value reaches 9 to 10 (approx. 50 ml/litre). The solution is maintained at 50°C for a further five minutes, allowed to cool and then centrifuged. The supernatant is discarded and the precipitate is dissolved in 1 litre 3% aqueous formic acid. The solution is centrifuged and the supernatant heated to 50°C with continual stirring. The glycoalkaloids are reprecipitated on addition of concentrated ammonia solution until the pH-value reaches 9 to 10. The solution is maintained at 50°C for a further five minutes as before, allowed to boil and then centrifuged. The glycoalkaloids precipitate is dried overnight at 50°C and then extracted with 100 ml boiling ethanol. The ethanolic solution is centrifuged and the supernatant dried at 50°C. This yields a fine powder.

The extract as prepared in Examples 1 and 2 will hereinafter be referred to as BEC-001.

The following example illustrates separation of the glycoalkaloids (II), (III) and the mono- and disaccharides of solasodine (I) from the BEC-001.

Example 3

The powder extract from Solanum sodomeum (BEC-001) was dissolved in an eluent containing acetonitrile/ Pic Reagent B_5/triethanolamine (83:17:0.1) adjusted to pH-value 2.7 to 3.0 with concentrated phosphoric acid, at a concentration of 0.1% (by weight).

50µl samples were applied to an injector (Model U6K Universal Injector*) and was chromatographed at ambient temperature on a 30cm x 3.9mm "Carbohydrate Analysis" or an "-NH_2" prepacked column*, average particle size 10µ m at a flow rate of 2 ml per minute (Model 6000A solvent delivery system*). A model 450 Variable Wavelength Detector*, with sensitivity set at 0.01 Aufs, was used and peak areas at 205 nm were recorded with a 10mV Omniscribe Recorder. (*Equipment obtained from Waters Associates Inc.)

Figure 1 shows a chromatogram of a sample of BEC-001. The retention times of peaks II, III and IV were 4.2 min., 6.25 min., and 9.55 min., respectively. These retentior times were identical to retention times of purified solasodine, solamargine and solasonine respectively.

Mass spectral analysis of the aglyconic solamargine and solasonine indicated that the aglycone was solasodine. The group of peaks designated I in Figure 1 represent mono- and diglycosides of solasodine. Mass spectral analysis of the aglycone from Peak II of Figure 1 confirmed its

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compositions as solasodin.

Suitable pharmaceutically acceptable salts of the compounds of formula I are, e.g. the hydrochlorides and hydrogen sulfates.

The compositions of the present invention may be used in the chemotherapeutic treatment of basal cell and squamous cell carcinomas, of skin inflammation, of mycotic infections such as tinea and ringworms, of viral infections such as warts, of haemorrhoids, of bacterial infections such as acne, and of non malignant dermatitis such as psoriasis, and for other cosmetic uses. They may also be used as adjuncts to topical antifungal and antibacterial preparations.

The carrier material employed in the compositions of the present invention may be an organic or inorganic inert carrier material suitable for internal (e.g. oral), external (e.g. dermal), or parenteral administration. Examples of such carrier materials include dimethylsulfoxide, water, lactose, starch, magnesium stearate, talc, gum arabic, gelatin polyalkylene glycols and petroleum jelly. The pharmaceutical preparations can be made up in a solid form, e.g., tablets, dragees, suppositories or capsules, or in liquid form, e.g., as solutions, emulsions, suspensions or aerosols. The pharmaceutical preparations may be subjected to customary pharmaceutical operations such as sterilisation and may contain adjuvants such as preservatives, stabilisers, wetting agents, buffers and salts for varying osmotic pressure.

Alternatively the steroid alkaloids may be formulated in suitable pharmaceutical vehicles for topical application e.g., as lotions, ointments or creams by incorporating them

in conventional lotion, ointment or cream bases, such as zinc oxide, alkyl polyether alcohols, cetyl alcohol, stearyl alcohol or polyethylene glycol 1000 monoacetyl ether (cetomacrogol). They may also be formulated as solutions in dimethylsulfoxide. Other solid forms include powders wherein the steroid alkaloids are incorporated in conventional powder bases such as starch or talc or jellies in which the base is, e.g. glycerol or tragacanth.

The following example illustrates formulations of BEC-001.

Example 4

The formulation was made up in cetomacrogol cream, zinc ointment or zinc cream. The concentrations of BEC-001 used were 2%, 4%, 10% and 20%.

The formulation was prepared by dissolving the extract in an equivalent weight of dimethylsulfoxide, with gentle heating and stirring. This was added to an appropriate weight of cream and the ingredients mixed until homogeneous using a domestic mixer. The prepared cream was dispensed into glass jars and stored in a refrigerator.

The following example illustrates toxicity tests on BEC-001 as prepared in Examples 1 and 2.

Example 5

Single Dose

The toxicity of a single ip dose of BEC-001 in mice is illustrated in Figure 2. It can be seen that the LD_{50}=30 mg/kg (mg BEC-001 per kg mouse body weight). Toxicity studies of glycoalkaloids extracted from Solanum tuberosum which contains mainly solanine were conducted by Patil et al., [Food and Cosmetics Toxicology 10,395 (1972)] who found

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an LD_{50} in mic (ip administration) of 32.3 mg/kg. Nishie t al. [Toxicol gy and Appli d Pharmacology 19,81 (1971)] reported an ip LD_{50} of 42 1.8 mg/kg in mice, whereas Gull et al., [Hortscience, 5,316 (1970)] found an ip LD_{50} of 75 mg/kg when the pure alkaloid was used. The values calculated from experimental data on BEC-001 agree closely with those of Patil et al., and Nishie et al. The postmortem examinations following administration of BEC-001, like those reported by Gull et al., revealed no well-defin d symptoms directly attributable to toxic effects of the glycoalkaloids. Patil et al., found that administration of an ip dose in mice of over 50 mg solanine/kg was lethal within 1 to 3 hr, but a dose of 10 mg/kg caused no deaths; this is in agreement with results found. It was found that the LD_{50} for gastric intubation in mice was 550 mg BEC-001 per kilogram, this is in close agreement with the value reported by Gull et al of 590 mg solanine per kilogram.

The LD_{50} of BEC-001 for rats was found to be 41 mg/kg.

Multiple Doses

The LD_{60} for mice by 14 ip administrations over 14 days (one injection per day) was 10 mg/kg.

The LD_{50} for rats by 8 ip administrations over 8 days (one injection per day) was 20 mg/kg.

Local Toxicity In Mice

The hair on the back of mice was clipped with electric clippers. The area of the hair removed was about 2.5 cm x 4.0 cm. Care was taken to avoid injury in a batch of 24 mic (batch A). Superficial skin injury using electric clippers was deliberat ly done on another batch of 24 mice

(batch B). The average w ight of mic was initially 40 g per mous (36-44g). Batch A was divid d into A_1 and A_2 of 12 mice each. Similarly batch B was divided into B_1 and B_2. Ten microlitres of dimethylsulfoxide was applied daily to the backs of mice in batches A_1 and B_1. Ten microlitres of dimethylsulfoxide containing 5% BEC-001 was applied to batches A_2 and B_2 daily. This study was continued for 16 weeks. At the end of the 16 weeks all mice grew normal hair and the skin appeared normal. A group of 5 mice of each batch was then sacrificed. Post mortem investigation revealed that there was no apparent gross abnormality of thoracic or abdominal contents.

Daily doses of approximately 12.5 mg BEC-001 per kg body weight appli d topically to intact or abraded skin of mice for 16 weeks did not produce any obvious adverse effects.

Side Effects

Patil et al., reported that solanine appeared to be a weak-to-moderate inhibitor of both specific and non-specific cholinesterase. Following small multiple doses of solanine, a quick inhibition followed by rapid recovery of serum cholinesterase was noted in the dog. Red-cell cholinesterase was not inhibited. It was speculated that while small doses of solanine may cause discomfort upon ingestion, repeated doses will have little noticeable effect resulting from acetylcholinesterase inhibition. Further studies by Patil et al., revealed that atropine appeared to be antagonistic to the toxicity of solanine.

In human experiments Ruhl [Archiv der Pharmazie 284,67 (1961)] indicated that an oral dose of 200 mg solanine

caus d hyp r sthesia, dr wsin ss, itchin ss in the neck region, and dyspnea: higher d ses caus d v mitting and diarrhoea. It has been found that an oral dose of 250 mg BEC-001 caused no such effects in a human subject.

The following example illustrates the effect of BEC-001 on sarcoma 180 activity in mice.

Example 6

A suspension of sarcoma 180 cells was taken from the peritoneal fluid of a host mouse and a known aliquot was injected ip into the experimental mice to produce sarcoma 180 activity in the ascitic fluid. BEC-001 was made up as a 5% stock solution in dimethylsulfoxide. The necessary control experiments were also conducted. Treatment of the mice was done by ip injections with appropriate quantities of BEC-001.

Figure 3 illustrates the inhibition of the activity of sarcoma 180 by BEC-001 (expressed as % survival of mice due to BEC-001). Mice inoculated with sarcoma 180 cells generally died in two to three weeks after inoculation. The criterion of survival was arbitrarily taken as eight weeks. This is 4 times normal time to death after inoculation of sarcoma 180 control mice untreated with BEC-001. It can be seen in Figure 3 that a single administration of BEC-001 has an ED_{50} of 10 mg/kg (i.e. activity of sarcoma 180 was inhibited in 50% of the animals of this dose).

Inhibition of the lethal effect of sarcoma 180 activity in mice was dependent on the number of doses of BEC-001. Figure 4 depicts the effect of the number of administrations of BEC-001 at a concentration of 8 mg/kg on the inhibition of the mice from this cancer type. Two doses

achieved 42% inhibition whereas with three and four doses almost complete inhibition was obtained.

Figure 5 illustrates the results obtained in the test on the inhibition of sarcoma 180 activity in the ascitic fluid of recipient mice using BEC-001. Five groups of twelve mice were used in the test. Curve Δ represents 1 injection of 8 mg/kg; Curve ● represents 2 injections of 8 mg/kg; Curve □ represents 3 injections of 8 mg/kg; Curve ▲ represents 4 injections of 8 mg/kg. The ip injections were done on consecutive days.

In summary; these studies indicate that BEC-001 is very effective in producing a highly significant inhibitory activity on the terminal cancer sarcoma 180 in mice. The efficacy and toxicity of BEC-001 depends on the route and type of application and is species dependent.

The following example illustrates the effect of BEC-001 on skin tumours.

Example 7

Studies of BEC-001 have shown exceedingly promising results in the treatment of skin cancers. Various preparations have been investigated ranging from crude plant extract (macerated fruit), BEC-001 in dimethylsulfoxide, BEC-001 in paraffin, BEC-001 . zinc ointment, BEC-001 in zinc cream, and BEC-001 in cetomacrogol. Studies done so far on a limited number of patients with skin tumours indicate that BEC-001 is effective to the types studied, viz, keratoses, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC).

Concentrations ranging from 0.1% to 50% BEC-001 have been studied on the skin in man. No apparent side effects

were observed. The studies indicate that the following formulation may be used to obtain satisfactory results.

BEC-001	4%
DMSO	5%
Cetomacrogol	91% all in W/W

One subject who originally had over 200 skin tumours has been using this preparation for over ten months and as far as can be determined has shown no ill effects.

One investigator has been applying 80 mg BEC-001 in the ointment form, twice daily for three months on himself and has encountered no apparent ill effects. An oral dose of 200 mg of BEC-001 did not affect the subject adversely. Biochemical (SNAC 20) and haematological (coulter screen, platelet count, differential W.C.C.) tests revealed ~~revealed~~ no significant changes due to BEC-001.

Two grams of the ointment are adequate to apply to 25 cm x 25 cm = 625 cm^2 area on the skin in man. This ~~this~~ corresponds to 1.6 mg glycoalkaloid per kg body weight for a person weighing 50 kg. This refers to topical application. The absorption kinetics of this drug through the skin is not known at present.

Oral toxicity studies in mice have shown that the LD_{50} is approximately 550 mg/kg.

Daily doses of approximately 12.5 mg BEC-001 per kg body weight applied topically to int or abraded skin of mice for 16 weeks did not produce any obvious adverse effects.

Since about early 1980, a series of clinical trials involving human patients of a pharmaceutical formulation of

a mixture of glycoalkaloids designated BEC-001, prepared as described in Example 4 have been carried out.

During the clinical trials, a number of patients exhibiting basal cell carcinomas (BCC's), squamous cell carcinomas (SCC's), solar keratoses and keratocanthomas (KA's) were treated with cream formulations of BEC-001. Histological tests, as well as biochemical and hematological studies were performed by appropriate departments at the Royal Brisbane Hospital, Queensland, Australia, to determine what side effects, if any, resulted from the BEC-001 treatment.

Through October 14, 1980, 19 BCC's were treated, 15 of which were histologically proven and four of which were clinically diagnosed. The BEC 001 cream was applied to the lesions twice daily at BEC-001 concentration levels of between 2% and 20%. Therapy lasted 4-16 weeks depending on the type of lesion and BEC-001 concentration.

Through such treatment, two histologically proven regressions and eight additional clinically proven regressions were obtaioned. Only one of the 19 BCC's continued to grow despite treatment.

In the same manner, a series of five histologically proven SCC's, plus one nodule which was diagnosed as grossly displastic were treated. Three of the SCC's histologically regressed, and a further one plus the displastic nodule, clinically regressed. The histology of the further one SCC showed a small pocket of malignant cells, indicating more treatment was required. The sixth lesion markedly decreased in size during treatment.

Two apparently normal patients were treated with the

cr am for six w eks. Biopsies taken from these patients show d no macroscopic or microscopic changes.

Treatment of clinically and histologically diagnosed disease having a duration from a few months to about 20 years with BEC-001 cream at concentration levels of 2-20% resulting in destruction of malignant cells in most cases with normal new nonmalignant cell growth and a complete absence of adverse side effects, except that mild pruritis surrounding the lesion being treated occured in a few cases. Pruritis disappeared with lowering of the concentration of the BEC-001 in the formulation.

A.T.

The claims defining the invention are as follows:

1. A pharmaceutical formulation containing at least on active ingredient selected from the group consisting of solamargine, solasonine and mono- and di-saccharides of solasodine and the pharmaceutically acceptable salts thereof together with a pharmaceutically acceptable carrier *excluding simple solutions or suspensions of said active ingredient in water or common organic solvents.*

2. A method for treatment or prevention of basal cell carcinoma, squamous cell carcinoma solar keratosis, keratocanthoma, tinea, warts, acne, psoriasis, haemorrhoids or skin inflammation ~~or other skin disorder~~, in a patient requiring said treatment or prevention, which method comprises administering to said patient an effective amount of a compound selected from the group consisting of solamargine, solasonine and mono- and di-saccharides of solasodine and the pharmaceutically acceptable salts thereof or of a pharmaceutical formulation as defined in claim 1.

3. The method of claim 2 wherein said compound or formulation is applied topically.

~~4. A process for extracting glycoalkaloids from~~ Solanum sodomeum comprising grinding parts of said plants subjecting the group plant matter to the action of at least one dilute acid and making the acid extract alkaline to precipitate said glycoalkaloids.

5. The process as defined in claim 4 wherein said acid is acetic acid or formic acid.

6. The process as defined in claim 4 or claim 5 comprising the steps of:

(i) grinding said plant material coarsely;

(ii) mixing said ground plant material with dilute acid to ~~form a first acid extract;~~

~~temperature is 50°C.~~

20. The process as defined in any on f claim 14 t 1e wh rein t p (f) and/~~or~~ (j), th pH is increased by addition of ammonia.

21. The process as defined in any one of claims 14 to ~~20 wherein said short period is 5 minutes.~~

DATED this NINTH day of MAY 1984

ARUBA (QLD.) PTY. LTD.

Patent Attorneys for the Applicant
SPRUSON & FERGUSON

A.T.

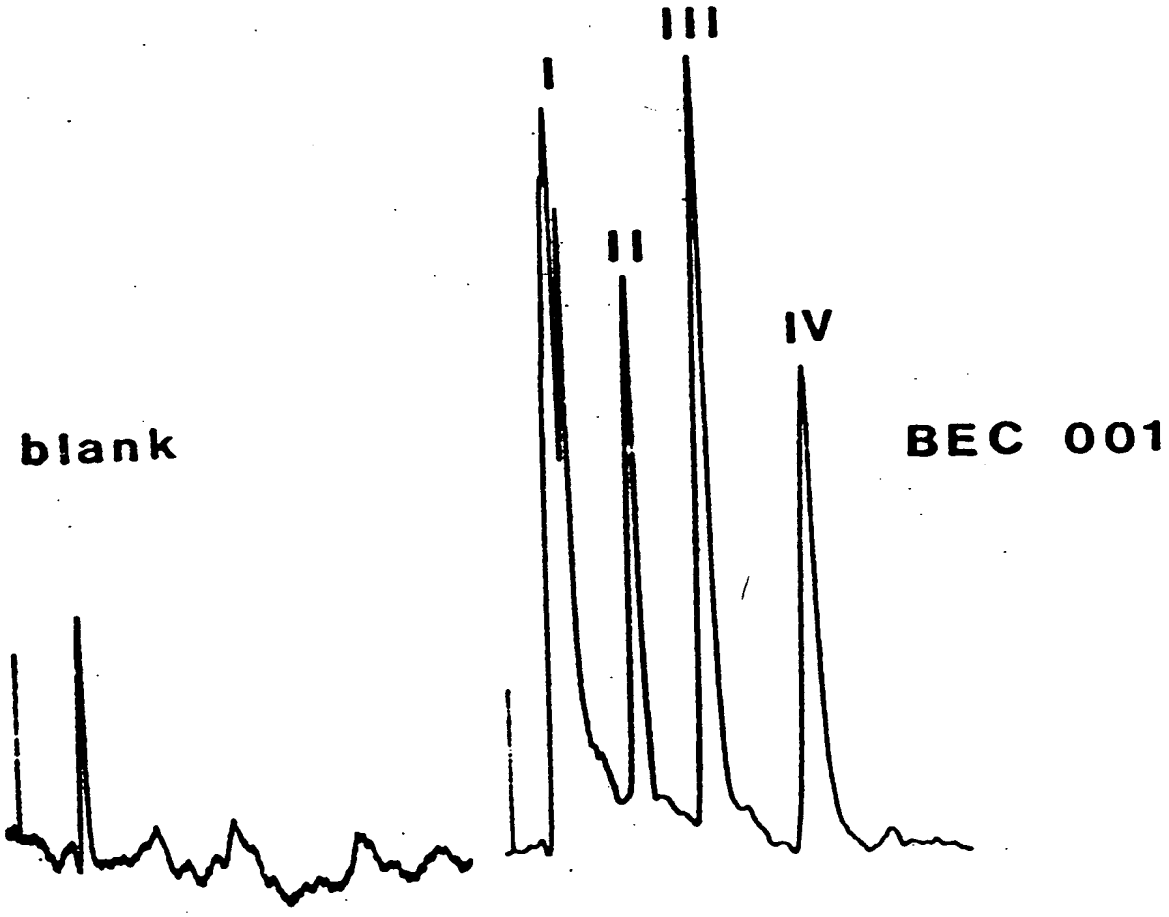
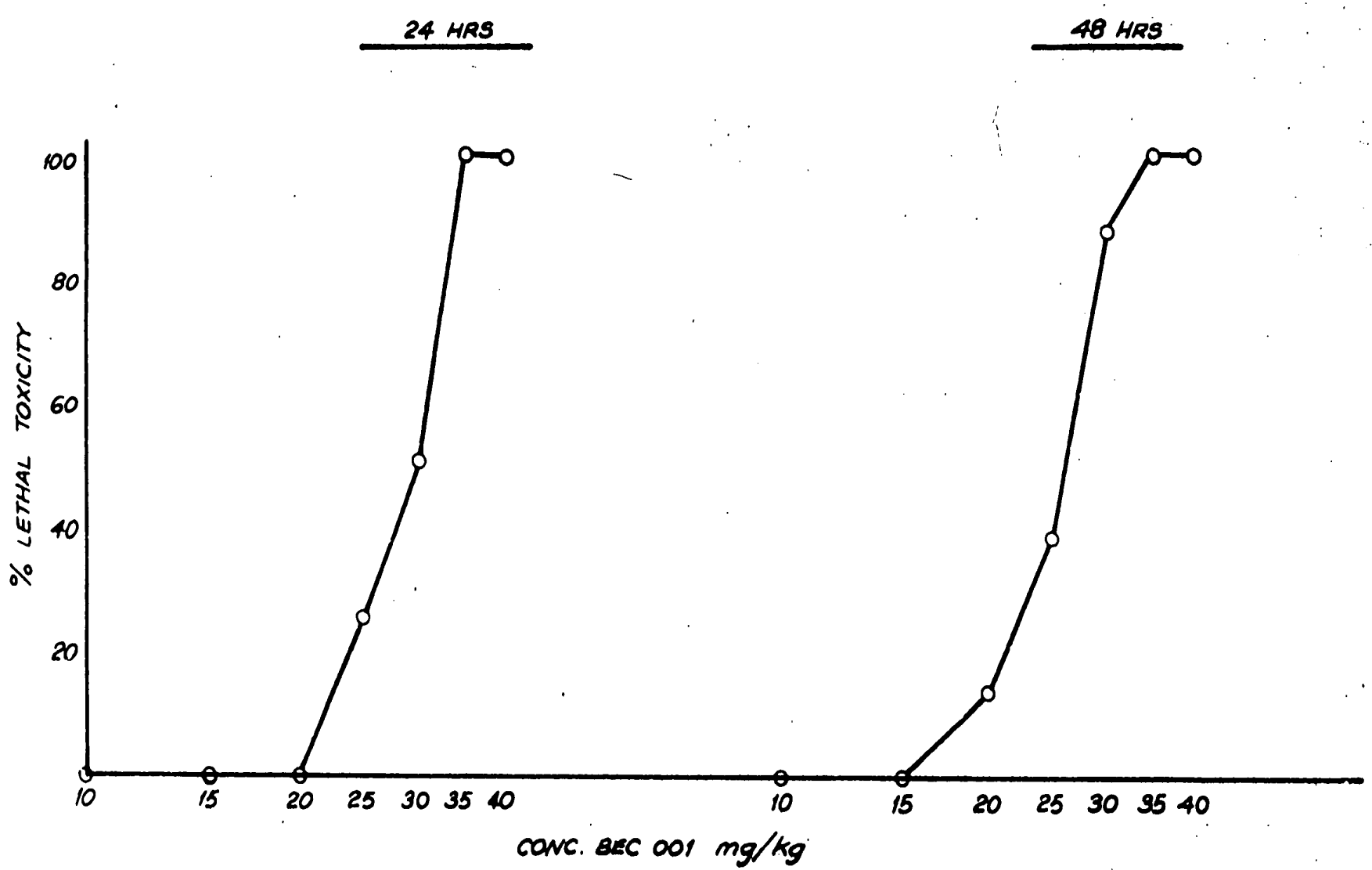


Figure 1


24 HRS
48 HRS
% LETHAL TOXICITY
100
80
60
40
20
10
15
20
25
30
35
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10
15
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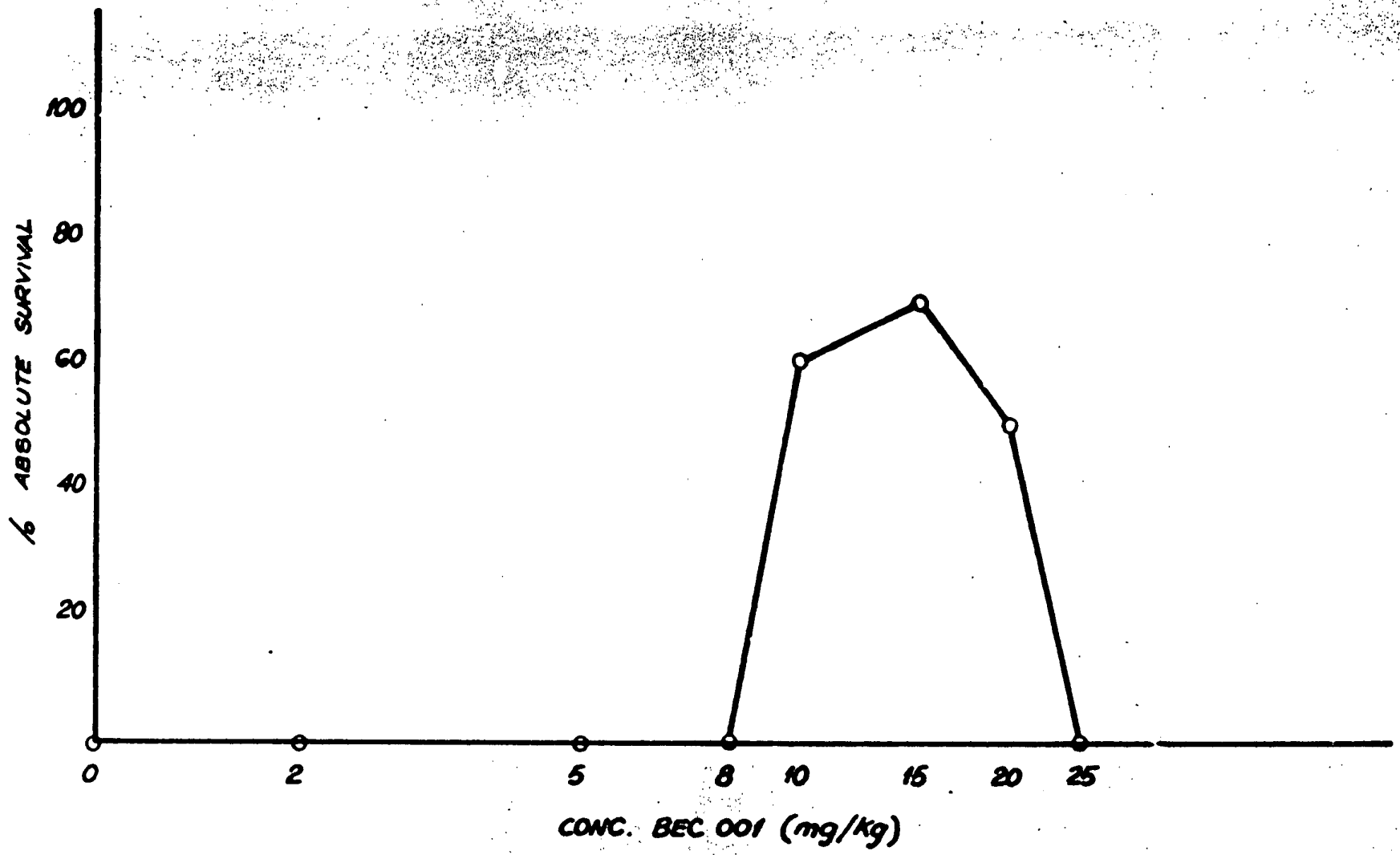
40
CONC. BEC 001 mg/kg

FIG. 2


% ABSOLUTE SURVIVAL
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40
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0
2
5
8
10
15
20
25
CONC. BEC 001 (mg/kg)

FIG. 3

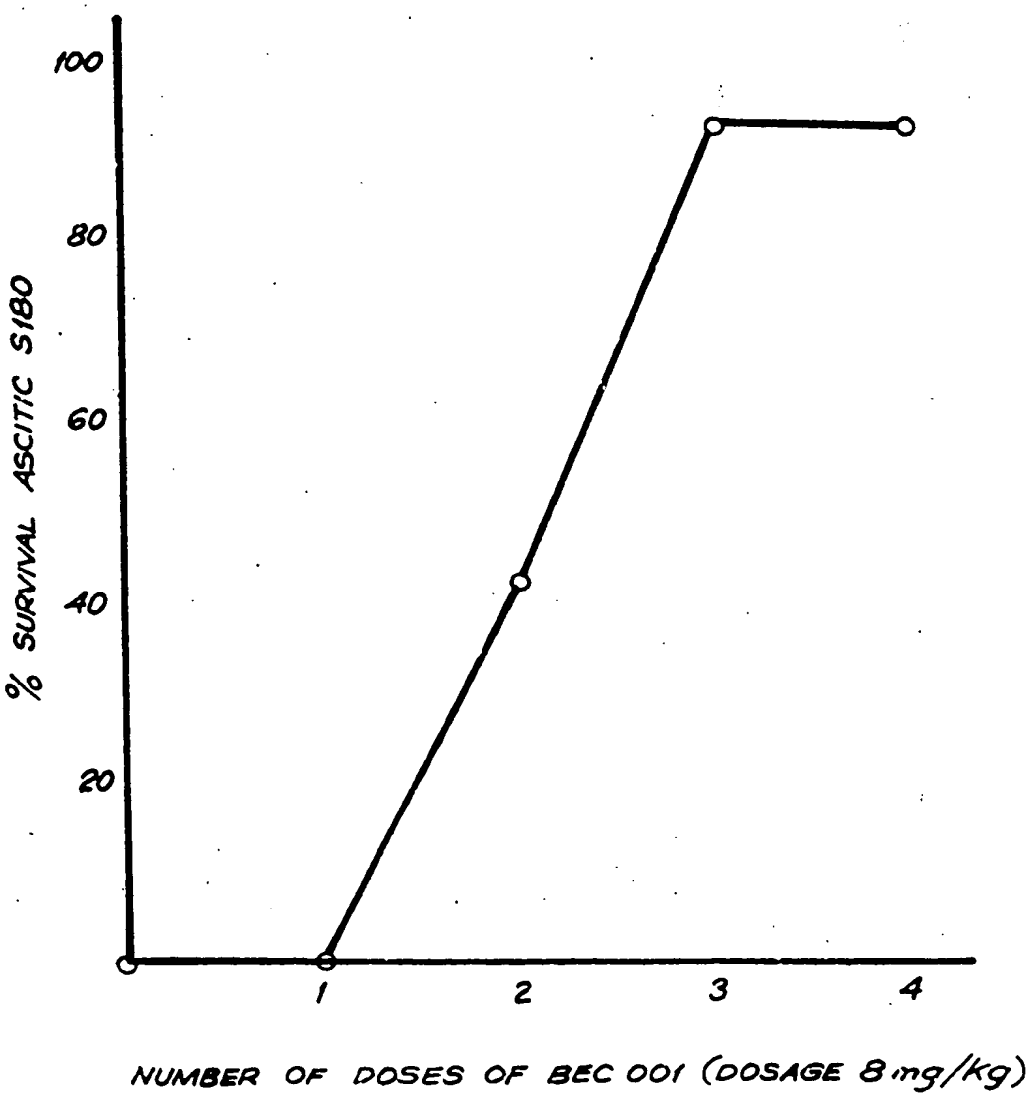
100
80
60
40
20
% SURVIVAL ASCITIC S180
1
2
3
4
NUMBER OF DOSES OF BEC 001 (DOSAGE 8 mg/kg)

FIG. 4

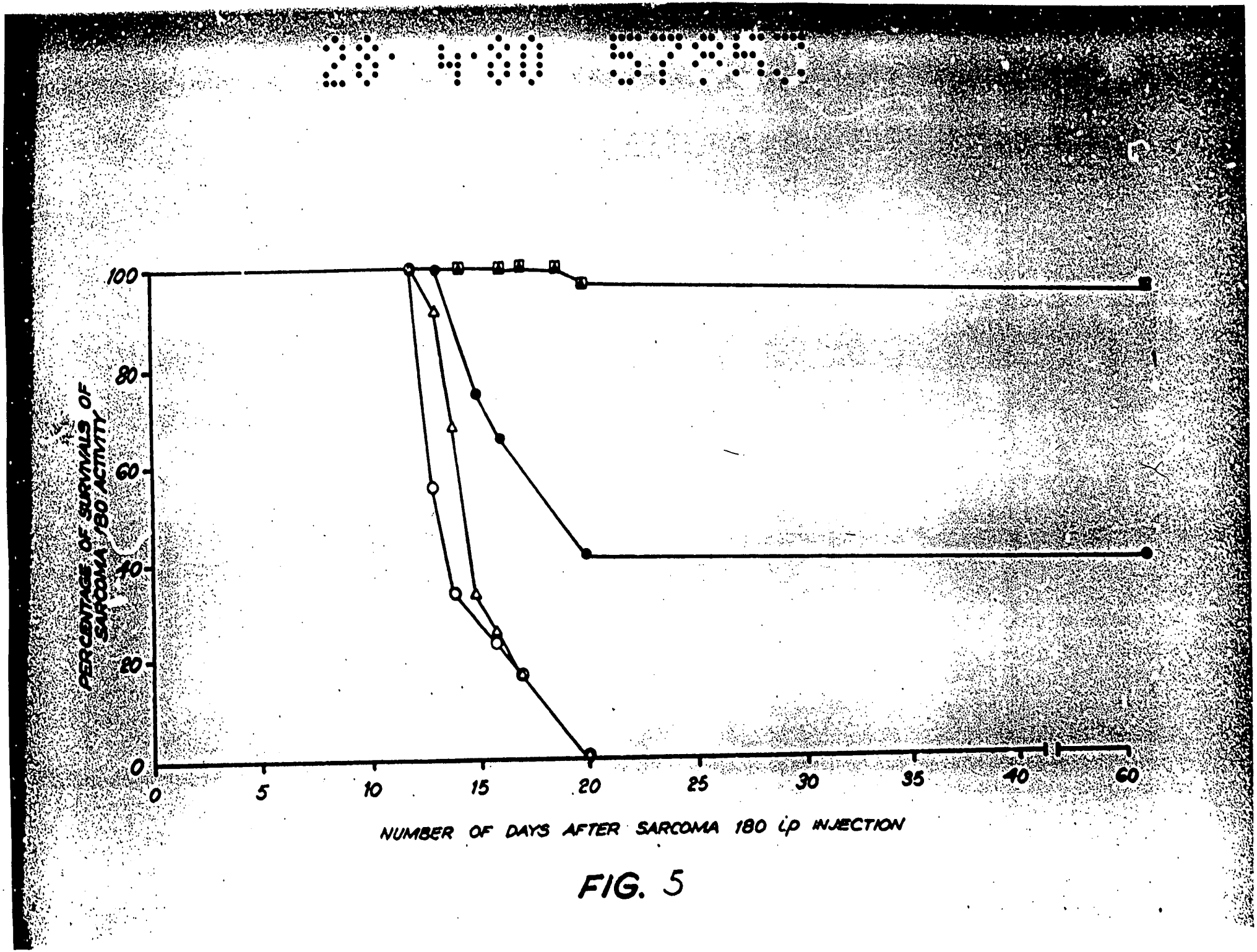
PERCENTAGE OF SURVIVALS OF SARCOMA 180 ACTIVITY
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
60
NUMBER OF DAYS AFTER SARCOMA 180 i.p. INJECTION

FIG. 5